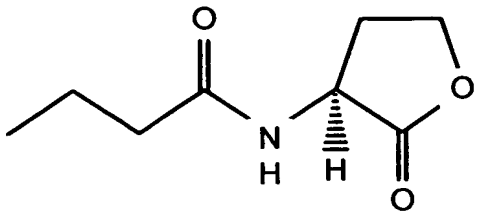
Quorum Sensing Signalling Molecules from
Pseudomonas aeruginosa

BHL (*N*-butanoyl L-homoserine lactone)



OdDHL (*N*-(3-oxododecanoyl) L-homoserine lactone))

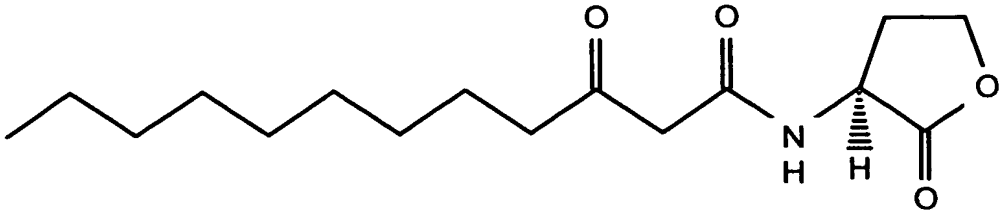
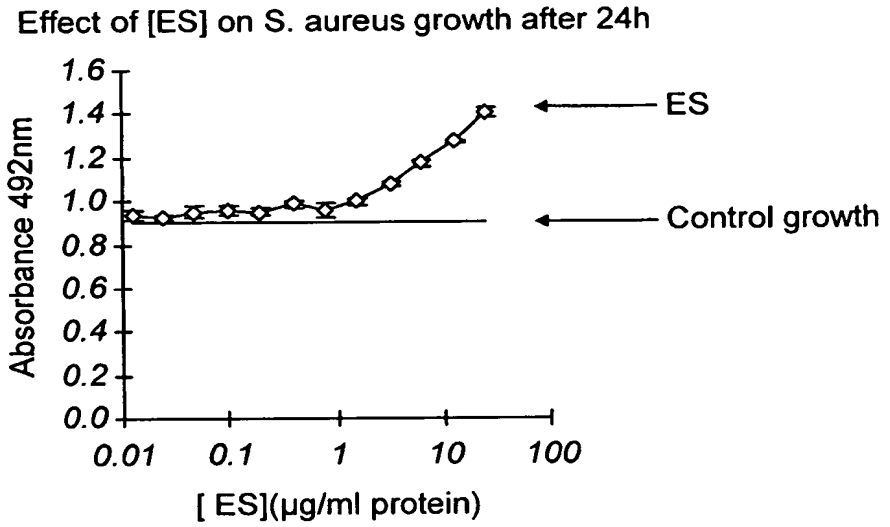
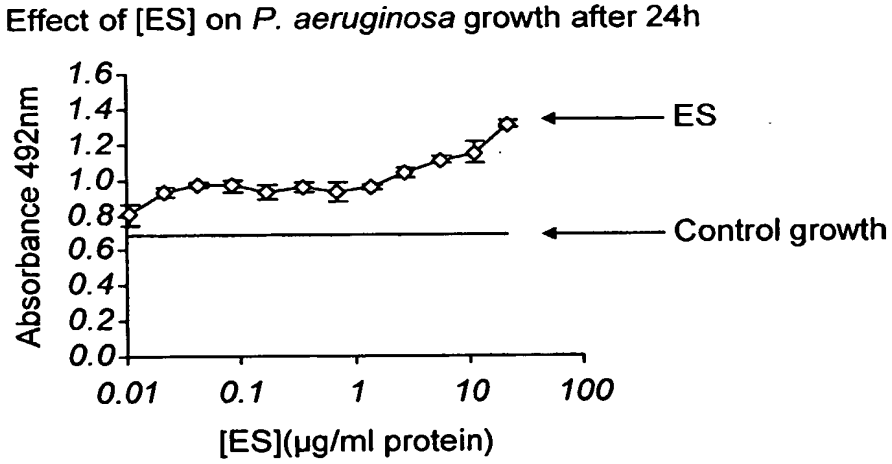


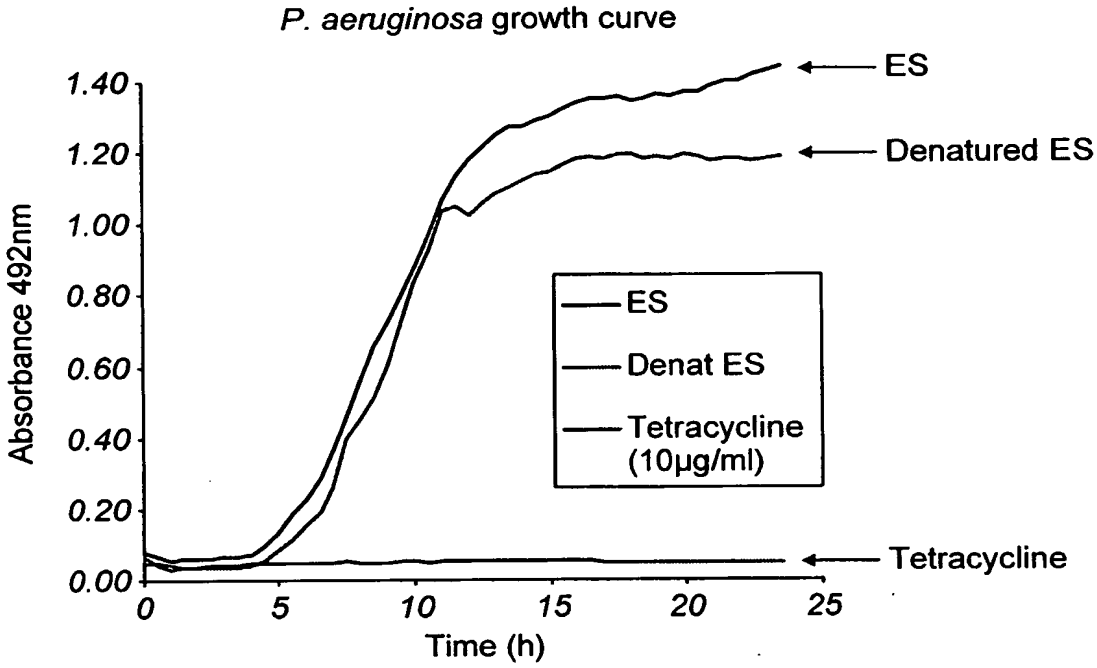
FIG. 1





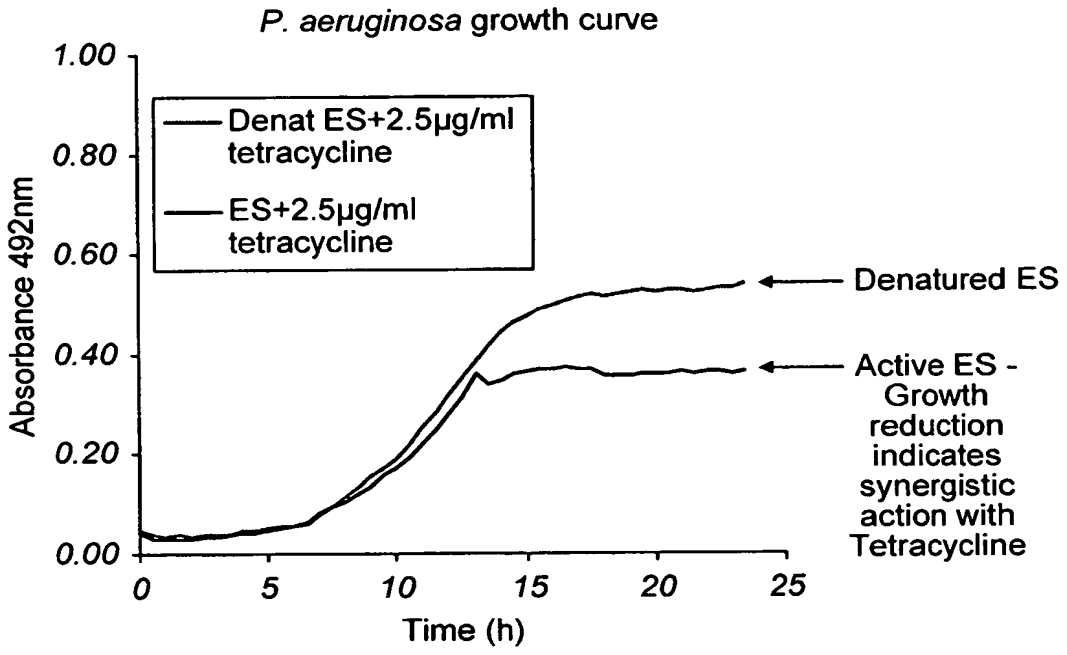
Effect of ES on planktonic growth of *P. aeruginosa* and *S. aureus*. Early log phase bacteria (~1000 organisms/well) were grown for 24h at 37°C in the presence of dilutions of ES in PBS in 96 well plates. Growth was measured by absorbance at 492nm.

FIG. 2



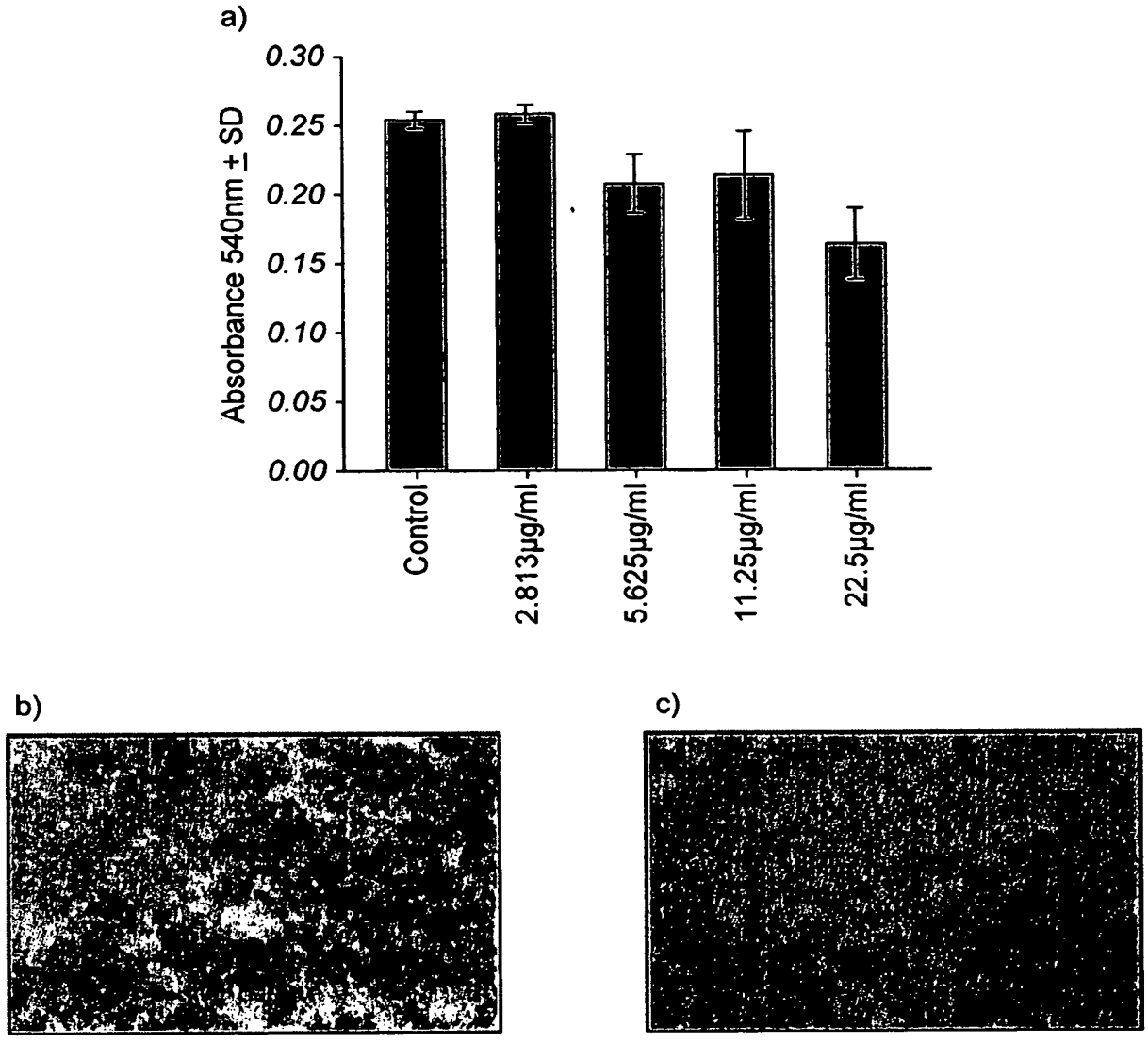
Effect of ES, denatured ES or Tetracycline on planktonic growth of *P. aeruginosa* over 24h measured by absorbance at 492nm. The higher absorbance after 24h in the presence of ES compared with the denatured form may be due to enzymatic stripping of sessile organisms from the well

FIG. 3



Effect of 2.5 µg/ml tetracycline with active and denatured ES on planktonic growth of *P aeruginosa* over 24h measured by absorbance at 492nm

FIG. 4



Biofilm formation in the presence of *L.sericata* ES

a) Action of *L.sericata* ES on *Staphylococcus aureus* biofilm, quantified by monitoring absorbance at 540nm of solubilised crystal violet bound to adherent cells. b,c) Action of ES growth of *Pseudomonas aeruginosa* biofilm on glass coverslips, visualised using Baclight™ stain b) represents control biofilm, after 20h growth. c) represents growth in the presence of *L.sericata* ES products (22µg/ml)

FIG. 5

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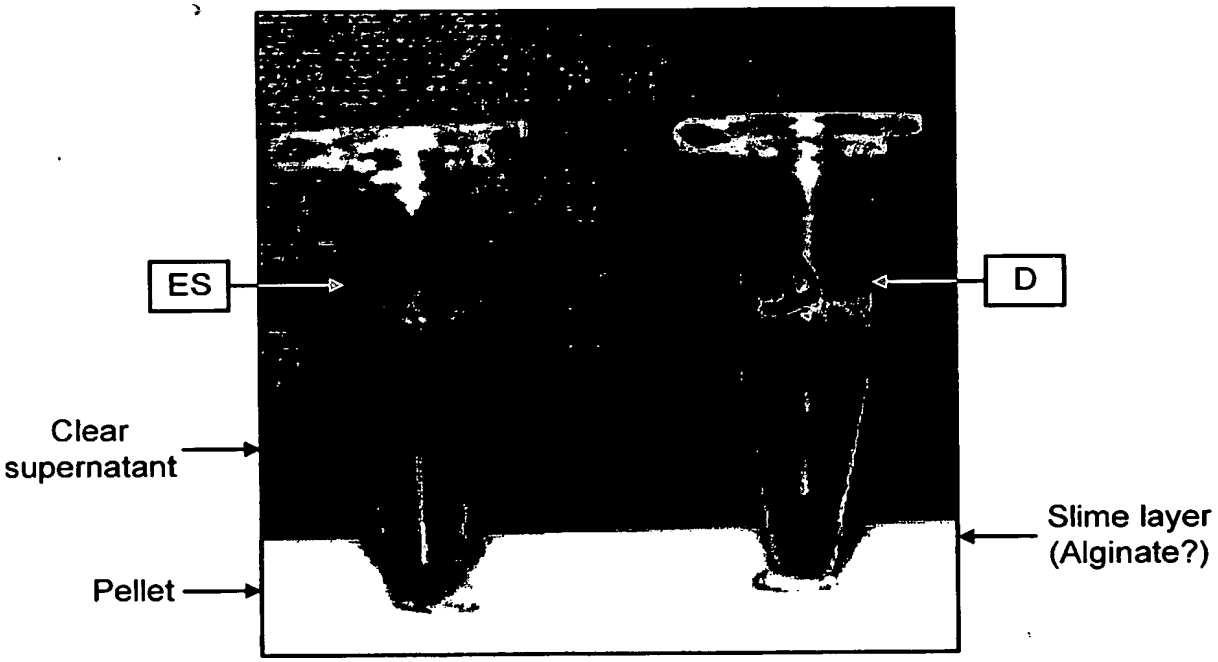
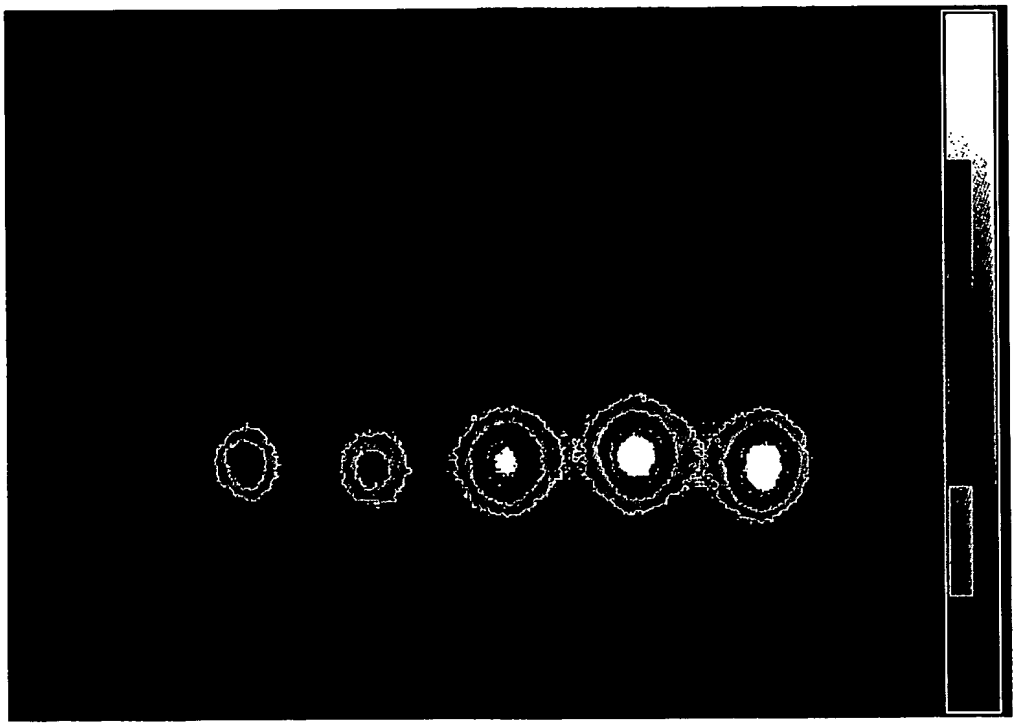


Pseudomonas aeruginosa 24h growth in biofilm producing conditions (100µl aliquots in a 96 well plate) containing active (ES) or denatured (D) *Lucilia sericata* secretions, collected together and microfuged (13,000 x g) for 10min. The slime layer gave ~500µg/ml glucose (equivalent) concentration on testing for carbohydrate using concentrated phenol sulphuric. The control (D) slime layer was subsequently removed by incubation (18h @ 37°C) with 2.5 µg of active ES.

FIG. 6

Degradation of BHL by *L. sericata* ES - Effect of boiling and of pre-incubation with inhibitors PMSF or APMSF



Thin layer chromatography of 1µl of 100µM BHL after incubation for 6h with *L sericata* ES (100µl - 120µg/ml total protein):

1. ES
2. Boiled ES
3. ES pre-incubated with APMSF (0.5mM)
4. ES pre-incubated with PMSF (2mM)
5. BHL control in phosphate buffered saline.

FIG. 7

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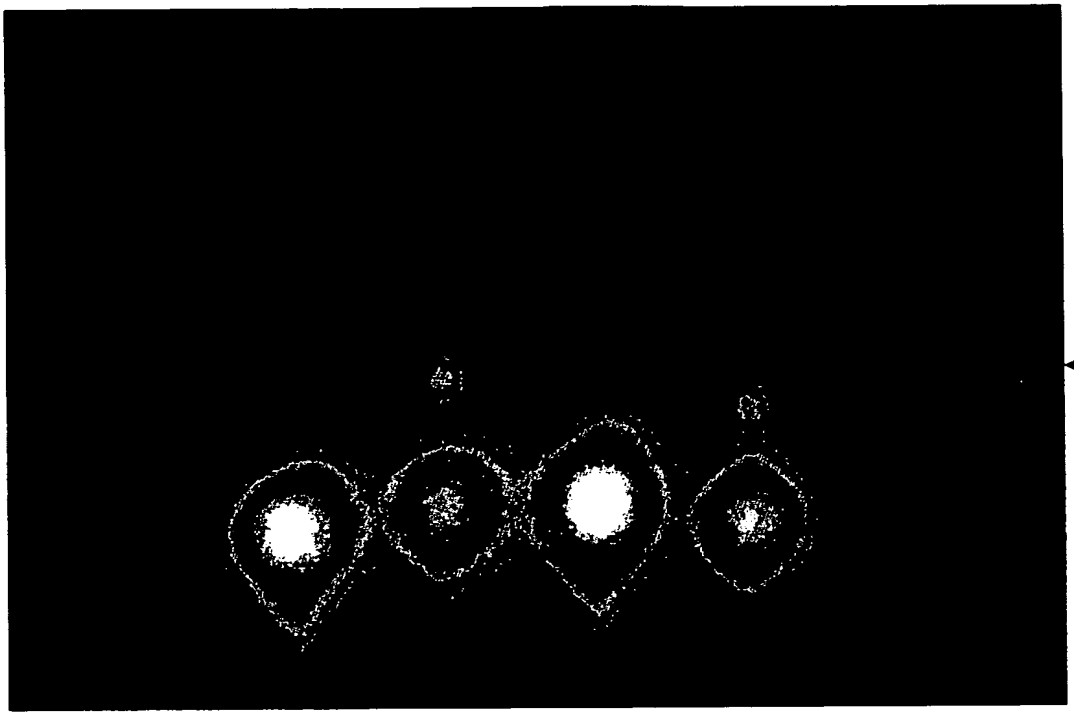
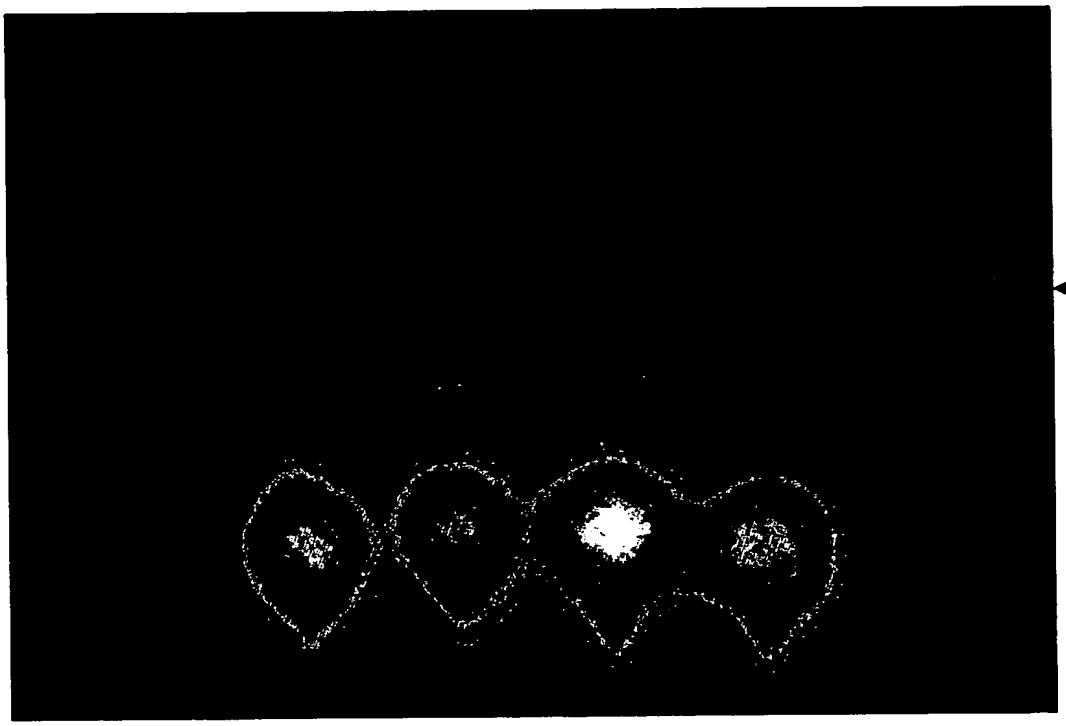
Degradation of OdDHL by *L. sericata* ES - Effect of boiling.



Thin layer chromatography of 1µl of 100µM OdDHL after incubation with *L sericata* ES (100µl - 120µg/ml total protein):
1. Boiled ES (t=0h)
2. Boiled ES (t=6h)
3. ES (t=0h)
4. ES (t=6h)

FIG. 8

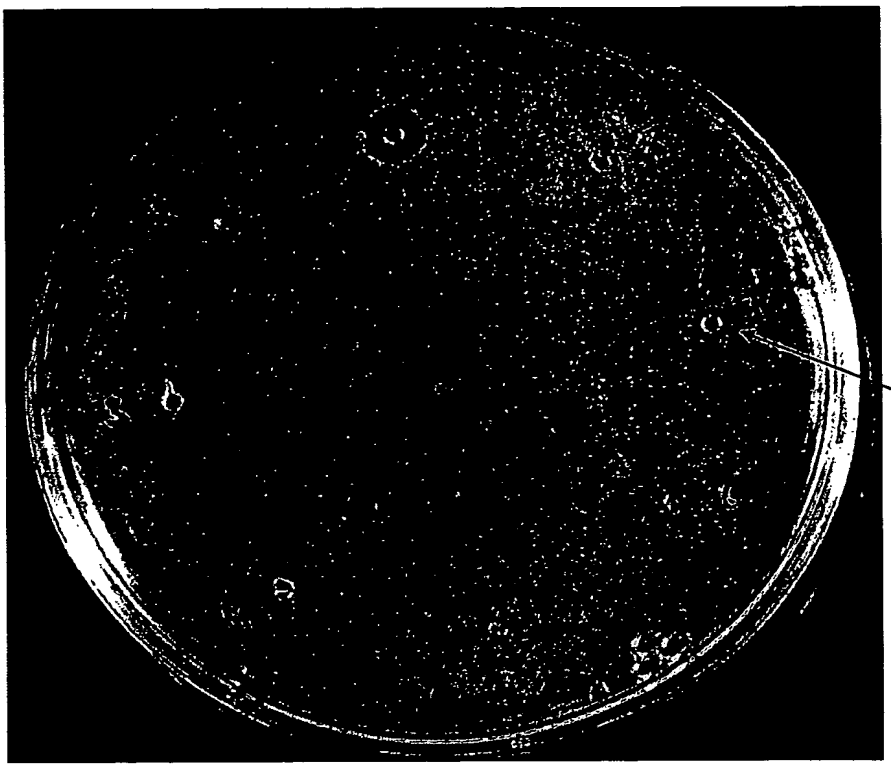
Degradation of OdDHL by *L. sericata* ES -
Effect of pre-incubation with PMSF and APMSF



Thin layer chromatography of 1µl of 100µM OdDHL after incubation with *L sericata* ES (100µl - 120µg/ml total protein):
1.ES pre-incubated with APMSF (0.5mM), t=0h
2.ES pre-incubated with APMSF (0.5mM), t=6h
3.ES pre-incubated with PMSF (2mM), t=0h
4.ES pre-incubated with PMSF (2mM), t=6h

FIG. 9

Growth inhibition of *E. coli* D31 by haemolymph from *L. sericata* larvae taken after induction by *Pseudomonas aeruginosa*.



Anti-microbial activity of *L sericata* haemolymph after challenge by *P. aeruginosa*. Standards of the anti-microbial peptide Cecropin B (Sigma) were used. Clear plaques in the E. coli D31 lawn indicated anti-microbial activity. Diameter measurements give an indication of the anti-microbial peptide concentration in the sample.

1. Cecropin B 100µg/ml
2. Cecropin B 10µg/ml
3. Cecropin B 1µg/ml
4. Cecropin B 0.1µg/ml
5. Control 4h
6. *P. aeruginosa* 4h
7. *P. aeruginosa* 48h
8. Control 48h

FIG. 10